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Resistance of Two Mediterranean Cold-Water Coral Species to Low-pH Conditions

Juancho Movilla ^{1,*}, Andrea Gori ¹, Eva Calvo ¹, Covadonga Orejas ², Àngel López-Sanz ¹, Carlos Domínguez-Carrió ¹, Jordi Grinyó ¹ and Carles Pelejero ^{1,3}

¹ Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas, Passeig Marítim de la Barceloneta, 37-49, Barcelona 08003, Spain; E-Mails: gori@icm.csic.es (A.G.); ecalvo@icm.csic.es (E.C.); alopez@icm.csic.es (A.L.-S.); cdominguez@icm.csic.es (C.D.-C.); grinyo@icm.csic.es (J.G.); carles.pelejero@icrea.cat (C.P.)

² Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Baleares (COB), Moll de Ponent s/n, Palma de Mallorca 07015, Spain; E-Mail: cova.orejas@ba.ieo.es

³ Institució Catalana de Recerca i Estudis Avançats, ICREA, Barcelona 08010, Spain

* Author to whom correspondence should be addressed; E-Mail: jmovilla@icm.csic.es (J.M.); Tel.: +34-93-230-9500; Fax: +34-93-230-9555.

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Abstract: Deep-water ecosystems are characterized by relatively low carbonate concentration values and, due to ocean acidification (OA), these habitats might be among the first to be exposed to undersaturated conditions in the forthcoming years. However, until now, very few studies have been conducted to test how cold-water coral (CWC) species react to such changes in the seawater chemistry. The present work aims to investigate the mid-term effect of decreased pH on calcification of the two branching CWC species most widely distributed in the Mediterranean, *Lophelia pertusa* and *Madrepora oculata*. No significant effects were observed in the skeletal growth rate, microdensity and porosity of both species after 6 months of exposure. However, while the calcification rate of *M. oculata* was similar for all colony fragments, a heterogeneous skeletal growth pattern was observed in *L. pertusa*, the younger nubbins showing higher growth rates than the older ones. A higher energy demand is expected in these young, fast-growing fragments and, therefore, a reduction in calcification might be noticed earlier during long-term exposure to acidified conditions.

Keywords: ocean acidification; cold-water corals; *Lophelia pertusa*; *Madrepora oculata*; Mediterranean Sea; aquaria experiment; calcification rate; porosity; microdensity

1. Introduction

Due to the absorption by the ocean of a major part of the anthropogenic CO₂ emitted to the atmosphere, the pH of global surface waters has already dropped by 0.1 units since the pre-industrial era [1–3]. In the case of the Mediterranean Sea, this pH decrease seems to have been greater than in the global ocean [4] and this area is actually considered one of the most sensitive regions to Ocean Acidification (OA) during the forthcoming years [5–8]. On the other hand, it is expected that zones characterized by naturally low carbonate concentration values such as high-latitude and deep-water ecosystems will be amongst the first to experience undersaturated conditions [9–11].

This would be the case of cold-water coral (CWC) communities, which are found in areas characterised by very low aragonite saturation state values (Ω_A) [12,13]. Therefore, studies evaluating the potential impact of OA on CWC populations are essential to determine the future of one of the most complex deep-sea habitats in the Mediterranean Sea.

However, unlike the numerous experiments with tropical or temperate corals, where OA has been suggested to have diverse ecological and physiological impacts [14–18], the responses of CWC to OA remain largely unexplored, with the few available studies showing contrasting results. For instance, the first short-term studies based on short incubations (between 24 h and 1 week-long) conducted on specimens of *Lophelia pertusa* and *Madrepora oculata*, found a reduction in the calcification rate ranging from 30% to 56% when the pH was dropped between 0.1 and 0.3 units [13,19,20]. On the contrary, in the most recent short-term (between 24 h and 21 days) and in the two medium-term experiments (between 6 months and 9 months) conducted to date with the same species, no effects were observed when rearing corals at pH values similar to those expected by the end of the century [13,20–22]. Thus, despite the different results observed in the first short-term experiments, the outcomes so far from medium-term experiments evidence that some CWC species could be well adapted to possible changes in the chemical conditions of seawater. It is important to remark that, in these previous studies on possible effects of OA in the development of CWC, coral growth was exclusively assessed through measurements of calcification, with no studies so far reporting on possible effects on coral skeletal microdensity or porosity.

In this context, we investigated the mid-term effect (6 months) of OA on the calcification rate, skeleton microdensity and porosity of two CWC species inhabiting deep Mediterranean waters, the branching corals *L. pertusa* and *M. oculata*. In this work, we compare our results with the two previous similar studies published with the same CWC species. We anticipate also possible long-term responses of these organisms based on experiments assessing the effect of OA on other Mediterranean corals, both temperate and CWC species.

2. Materials and Methods

2.1. Specimen Collection and Experimental Setup

Colonies of *L. pertusa* and *M. oculata* were collected at 250 m depth in the Cap de Creus canyon (NW Mediterranean Sea) in July 2006 and September 2007 by means of the ROV “Phantom HD2 + 2” and the submersible “JAGO” (GEOMAR) respectively, on board of the Research Vessel “García del Cid”. Coral specimens were kept in aquaria with 50 µm filtered running natural seawater at salinity of 38, temperature of 12 °C and in complete darkness as described by Olariaga *et al.*, [23]. A mixed diet including frozen *Cyclops*, *Mysis* and *Artemia* (Ocean Nutrition TM) were supplied 5 days a week. At the beginning of the experiment, 24 nubbins of *L. pertusa* (2–5 polyps) and 36 of *M. oculata* (8–20 polyps) were selected and randomly distributed and incubated together into six 30 litres aquaria subject to two pH treatments (8.10 and 7.81 for control and acidified conditions, respectively; 3 replicates per treatment). See Bramanti *et al.* [24] for further details on the experimental setup. Discrete analyses of total alkalinity (TA) by potentiometric titration [25,26] and seawater pH by spectrophotometry [27] were carried out periodically. The rest of the carbonate system parameters were calculated using the CO2calc software (v1.0.30 USGS).

2.2. Skeletal Measurements

Skeletal growth of all coral nubbins was assessed every two months by means of the buoyant weight technique [28,29], using a 0.1 mg resolution balance (Mettler Toledo AB204 SFACT) and a YSI-30M probe to monitor temperature and salinity. The net buoyant weight (BW) of the corals was transformed to dry weight (DW) using the specific value of the aragonite skeleton density for each species previously determined (see below). To evaluate possible differences between the pH treatments, data were normalized with respect to the skeletal DW of the nubbins at the beginning of each sampling period. Calcification rates were calculated using an exponential growth function and results are expressed as mg of CaCO₃ increase per gram and day.

One nubbin of each species and aquarium was randomly selected at the end of the experiment to estimate specific skeleton microdensity and porosity following the technique described in Bucher *et al.*, [30]. Coral nubbins were dipped in sodium hypochlorite during 2 days to remove the organic matter and washed with distilled water afterwards. BW and DW of each sample were recorded before and after the inclusion in molten paraffin wax. In both cases, the BW was measured in distilled water at 20 °C with specific gravity of ~1.00 g cm⁻³.

2.3. Statistical Analysis

A one-way ANOVA was used to examine differences between treatments in skeleton microdensity and porosity. Repeated-measures two-way ANOVA was used to test potential variations in skeletal growth rates between treatments and aquaria over time (aquarium was considered as a random factor nested within treatment). Normality and homoscedasticity were examined using the Kolmogorov-Smirnov and Levene tests, respectively. Statistical analyses were performed using JMP 9.0.1 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

The seawater CO₂ system conditions of both treatments were constrained by periodical pH_T and TA laboratory measurements and results are shown in Table 1 (all values are expressed as mean ± SD). In the control treatment, pH_T and TA (8.092 ± 0.022 pH units and 2540 ± 13 μmol kg^{−1}, respectively) were comparable to those observed in the sampling area at similar depths during a cruise carried out in 2011 (8.084 ± 0.017 pH units and 2559 ± 16 μmol kg^{−1}, respectively). The calculated χCO₂ (mole fraction of CO₂ in dry air) and Ω_A values were 389 ± 24 ppm and 2.8 ± 0.1 , respectively. For the acidified treatment, a decrease of pH_T of ~0.3 units (7.808 ± 0.031 pH units) at similar TA values (2541 ± 12 μmol kg^{−1}) caused an increase in χCO₂ up to 821 ± 61 ppm and a decrease of Ω_A to 1.6 ± 0.1 . Temperature and salinity remained constant throughout the experiment (12.3 ± 0.3 °C and 37.6 ± 0.1 , respectively). Undersaturation conditions with respect to the aragonite were not reached at any time.

Table 1. Parameters of the seawater carbonate system in the aquaria for each treatment. Total alkalinity, pH_T, salinity and temperature were used to calculate all the other parameters using the CO2calc software (USGS). For the four measured parameters we report the values as mean ± SD (N = 4) and range (in brackets). All other calculated parameters are expressed as mean ± SD (N = 4).

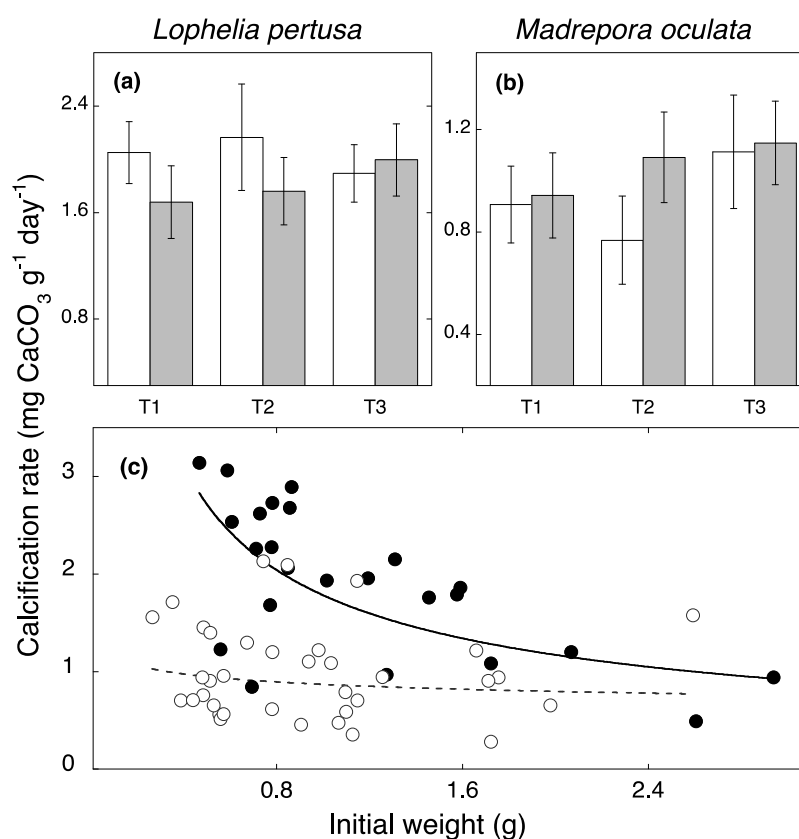
Measured parameters								
Treatment	pH _T		TA		Sal		T	
Control	8.092 ± 0.022		2540 ± 13		37.6 ± 0.1		12.3 ± 0.3	
	(8.061 – 8.112)		(2521 – 2551)		(37.5 – 37.8)		(11.9 – 12.5)	
High-CO ₂	7.808 ± 0.031		2541 ± 12		37.6 ± 0.1		12.3 ± 0.2	
	(7.787 – 7.854)		(2524 – 2551)		(37.5 – 37.7)		(12.0 – 12.5)	
Calculated parameters								
Treatment	pCO ₂	χCO ₂	DIC	[CO ₂] _{aq}	[HCO ₃ [−]]	[CO ₃ ^{2−}]	Ω _C	Ω _A
Control	384 ± 23	389± 24	2286 ± 16	15.4 ± 0.9	2087 ± 20	184 ± 7	4.3 ± 0.2	2.8 ± 0.1
High-CO ₂	809 ± 61	821 ± 61	2420 ± 18	32.4 ± 2.6	2283 ± 21	105 ± 7	2.5 ± 0.2	1.6 ± 0.1

Notes: pH_T = pH in total scale; TA = total alkalinity (μmol/kg-SW); Sal = salinity; T = temperature (°C); pCO₂ = partial pressure of CO₂ of air in equilibrium with seawater (ppm); χCO₂ = mole fraction of CO₂ in dry air (ppm); DIC = dissolved inorganic carbon (μmol/kg-SW); [CO₂]_{aq} = CO₂ concentration in seawater (μmol/kg-SW); [HCO₃[−]] = bicarbonate ion concentration (μmol/kg-SW); [CO₃^{2−}] = carbonate ion concentration (μmol/kg-SW); Ω_C = saturation state of seawater with respect to calcite; Ω_A = saturation state of seawater with respect to aragonite.

Regarding skeletal measurements, microdensity and porosity for *L. pertusa* were 2.81 ± 0.01 g cm^{−3} and $53.3 \pm 2.5\%$, respectively and for *M. oculata* 2.78 ± 0.01 g cm^{−3} and $42.0 \pm 1.8\%$, respectively (mean ± SE, N = 6). No significant differences between treatments were observed in microdensity or in porosity at the end of the experiment, neither for *L. pertusa* (ANOVA, $F_{1,5} = 0.978$, $p = 0.396$ and ANOVA, $F_{1,5} = 0.733$, $p = 0.440$, respectively) nor for *M. oculata* (ANOVA, $F_{1,5} = 0.492$, $p = 0.522$ and ANOVA, $F_{1,5} = 1.727$, $p = 0.259$, respectively). Mean calcification rate of *L. pertusa* reared under control conditions was double than *M. oculata* (2.040 and 0.922 mg CaCO₃ g^{−1} day^{−1}, respectively).

These growth rates are comparable to those previously measured on the same CWC species using different techniques [19–22]. However, the average growth rate of *L. pertusa* observed in the experiment of Form and Riebesell [13] was about one order of magnitude lower than in previous works, probably due to the lower incubation temperature used by these authors (7.5 °C) compared to other studies (between 10 and 13 °C). No significant differences between treatments were observed among the different sampling times for both species (ANOVA, $F_{4,18} = 0.110$, $p = 0.739$ for *L. pertusa* and $F_{4,30} = 0.138$, $p = 0.406$ for *M. oculata*; Figure 1a,b). Our results are in accordance with two previous mid-term studies assessing the effects of OA on these same species, where no differences were observed after 6 months in *L. pertusa* [13] or 9 months of exposure in *L. pertusa* and *M. oculata* [21]. This suggests that, at least at mid-term, CWC are able to counteract the *a priori* more negative environment that lowered-pH oceans should create for calcifying organisms. Regarding possible effects of time on calcification, in contrast with changes observed by Maier *et al.* [21], no significant differences were detected neither for *L. pertusa* (ANOVA, $F_{2,17} = 0.052$, $p = 0.649$) nor for *M. oculata* (ANOVA, $F_{2,29} = 0.614$, $p = 0.254$) throughout the present experiment.

Figure 1. Skeletal growth rates of (a) *Lophelia pertusa*; and (b) *Madrepora oculata* under control (white bars; $pH_T \sim 8.10$ units) and acidified conditions (grey bars; $pH_T \sim 7.81$ units) taking as a reference the weight at the beginning of each sampling period (T1, T2 and T3 represents 62, 118 and 182 days, respectively; results expressed as mean \pm SE; $N = 12$ in *L. pertusa* and 18 in *M. oculata*); (c) Correlation between the initial weight of the nubbins and their calcification rate at the end of the experiment computed for the whole duration of the experiment (182 days), for *L. pertusa* (solid line, black dots) and *M. oculata* (dashed line, white dots).



However, it is important to remark that a different growth pattern was observed between both species depending on the initial size of the nubbins. While all specimens of *M. oculata* showed similar growth rates (calcification rates measured for the whole duration of the experiment), a correlation between the calcification rate and the initial weight of the fragments was observed in *L. pertusa* (Figure 1c), where smaller nubbins exhibited greater calcification rates than those with a higher initial weight. This kind of species-specific response could be probably due to the different kind of fragments used for each species. Most of the *L. pertusa* nubbins used in our experiment had 2 to 3 polyps per nubbin, being assumable that a lower initial weight could be generally related to smaller and, therefore, younger polyps. In contrast, given to the smaller size of *M. oculata* polyps, each nubbin had to include between 8 and 20 polyps in order to detect potential changes by means of the BW technique. As a result, it was not possible to assess the different growth between small and large polyps in this species, given the coexisting combination of polyps in our selected nubbins, including nubbins with many small polyps with nubbins with few large polyps. At the end, the growth vs. initial nubbin weight plot (Figure 1c) in this species displayed similar growth rates for all fragments, though this result was not fully informative given these circumstances.

A similar growth pattern to that showed in this work by the Mediterranean *L. pertusa* has been previously described in *L. pertusa* from the North Atlantic [19] as well as in *Desmophyllum dianthus* from the Mediterranean [31]. In both studies, the reduction in calcification rate was greater in fast-growing young polyps compared to the larger and older ones. In other Mediterranean coral species such as the temperate *Cladocora caespitosa* and *Oculina patagonica*, the faster growing colonies were more affected by a decrease in pH [32], although in this study, the intraspecific genetic variability could be key in explaining the wide variety of responses observed. In any case, the regulation of the internal pH at the site of calcification has been described as an energy demanding process [33–35], and under lowered-pH conditions, it is expected that the energetic requirements for calcifying should be greater in coral fragments showing faster growth rates. Therefore, further studies conducted through even longer experimental time and focusing on young polyps could provide a better understanding of the threats that future OA will pose for CWC species, due to their role in maintaining the structure of the reef and their apparently higher sensitivity to acidified conditions.

4. Conclusions

Our data show a low sensitivity of *L. pertusa* and *M. oculata* to near future acidification in the Mediterranean Sea at mid-term. No apparent pH-driven effects were observed in the skeletal growth rate, microdensity and porosity of both species compared to control conditions after 6 months of exposure. However, we found a high intraspecific variability in the calcification rate among different colony fragments of *L. pertusa*, with small (and younger) nubbins showing faster skeletal growth rates. As has been observed recently with both temperate and CWC species, fragments showing higher growth rates are likely to show also greater sensitivity to low pH conditions and it is essential to include them in future long-term experiments to better understand the community-level response to OA.

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Conflicts of Interest

The authors declare no conflict of interest.

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